need not be stoichiometric with each thin filament, nor does nebulin regulate filament lengths in a binary, all-or-nothing fashion. As the numbers of nebulins per sarcomere are decreased, the local concentration of tropomodulin is diminished, leading to lower effective capping activity, increased actin assembly and uniformly longer thin filaments (Figure 1B). This model is also consistent with the dependence of thin filament length on tropomodulin concentrations [15–17].

How can the question of whether nebulin acts as a molecular ruler or a cap locator be resolved? The gold standard of proof for a molecular ruler is generally believed to be genetic replacement with ruler molecules of altered lengths, leading to corresponding predicted changes in filament lengths [5]. But this approach would not rule out a concentrative mechanism in which the M1M2M3 domain regulates tropomodulin capping at altered distances. Instead, the template function (the modular repeats) and the cap locator function (the M1M2M3 domain) must be physically dissociated. If the M1M2M3 domain were to be mislocalized, then tropomodulin capping would be increased in this new location and filament lengths would change correspondingly.

The muscle sarcomere is a complex, highly ordered structure, but the molecules that make up the elements of this structure are dynamic and their length control cannot be explained by a simple ruler mechanism. A static ruler mechanism relying on precise stoichiometries cannot be used to determine sizes of dynamic polymers. This is because to allow for variations in filament length, cells would have to make multiple rulers of assorted lengths. Instead, cells have complex layers of regulation to allow for the reuse of their polymer building blocks in countless combinations and amounts to achieve many different outcomes. In the case of muscle, nebulin is one part of the combinatorial regulatory process that defines the precise filament lengths required for physiological functions. Determining the molecular mechanisms by which muscle filament lengths are regulated will provide new paradigms to explain macromolecular size control. Dissecting the role of nebulin in regulating filament lengths is a good place to start.

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Developmental Biology: Micromanaging Muscle Growth

Much remains to be learnt about the *in vivo* function of specific microRNAs. Recently, the conserved microRNA *miR-1* has been found to be essential for *Drosophila* development. *miR-1* mutants die during the rapid larval growth phase with severe muscle defects.

Michael V. Taylor

In the last few years, the widespread prevalence of microRNAs encoded in the genomes of organisms ranging from the nematode worm, *Caenorhabditis elegans*, to humans has been discovered. MicroRNAs are short RNA molecules, around 22 nucleotides in length. As a group, they can control gene expression by two routes: promotion of mRNA

degradation or inhibition of mRNA translation. Their discovery has rekindled interest in the regulation of development by post-transcriptional control mechanisms.

The first microRNA, *lin-4*, was described in 1993 [1] in a 'forward' genetic screen in *C. elegans* for genes involved in the regulation of stage-specific events during development. This laid down an early marker that a microRNA could have an interesting and specific role in development. In the last few years, there has been rapid progress in many facets of microRNA biology, all thoroughly documented in recent reviews [2–6]. Current estimates indicate that animal genomes encode several hundred microRNAs. Moreover, recent computer-based searches for microRNA targets have led to suggestions that microRNAs might regulate the expression of as many as 20–30% of all genes [7,8]. However, much about their *in vivo* roles remains to be determined and it is striking that only a handful of microRNAs have been analysed *in vivo* using loss-of-function mutations. A new example has been described recently by Sokol and Ambros [9] who 'knocked-out' a conserved microRNA, called *miR-1*, in the fruitfly *Drosophila* and found an intriguing phenotype in larval development.

To knock out *Drosophila miR-1* (*DmiR-1*), Sokol and Ambros [9] followed the still relatively rarely used method for targeted deletion in *Drosophila* developed in Kent Golic's lab [10]. This identified *DmiR-1* as an essential gene required for viability. *DmiR-1* knock-outs die as small, second instar larvae. There is growth arrest, the larvae become increasingly lethargic and compromised in movement, and larval body architecture deteriorates, collapses and flattens [9]. How does the observed phenotype arise, and what clues does this give about the function of *DmiR-1*?

To answer these questions the authors explored the effect of *DmiR-1* on muscle, mainly because of its striking and specific expression pattern. *DmiR-1* is expressed initially throughout the embryonic mesoderm, and subsequently in the different types of larval muscle as they differentiate [9]. After hatching, the muscles grow substantially during the three larval instars; however, the expression of *DmiR-1* during these stages has not yet been determined. It has recently emerged that a specific pattern of expression, like that of *DmiR-1*, is not unusual for a microRNA. A trail-blazing study published earlier this year shows that 78 out of 115 conserved microRNAs tested are expressed in specific patterns [11]. Such descriptions have only recently become possible by using Locked Nucleic Acid modified oligonucleotides [12]. This technique for visualising microRNA expression patterns will have a revolutionary impact on the field, because most microRNAs have been identified independently of their function. Expression patterns will, therefore, provide important clues as to the functions of a given microRNA.

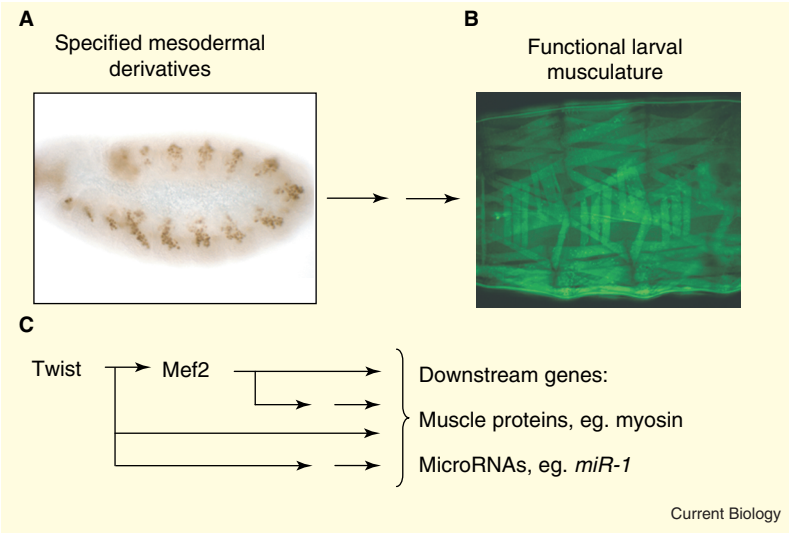

Figure 1. The developmental programme of the larval musculature in *Drosophila*.
(A) Progenitor cell populations for the different mesodermal derivatives, including the various muscle-types, are defined by approximately a third of the way through embryogenesis. At this stage, a lateral view of an embryo stained for the Twist protein shows some of these segmentally repeated progenitors. (B) At the end of embryogenesis, all elements of the larval musculature are in place. These muscles subsequently grow substantially and rapidly during larval life. Part of the body wall musculature in a larva is shown visualised with myosin–GFP. (C) Two key regulators of muscle development, the transcription factors Twist and Mef2, orchestrate the events of muscle differentiation. Arrows indicate that Twist activates *mef2* gene expression, and that both transcription factors activate a range of target genes either directly or indirectly to control muscle development. These targets include the genes that encode muscle proteins, e.g. myosin, tropomyosin, and now recent work indicates that microRNAs should also be considered amongst them [9].

DmiR-1 is expressed in muscle, so does it function there? Sokol and Ambros [9] could not detect muscle defects in *DmiR-1* mutant embryos, despite the specific expression during embryogenesis, and so turned their attention to larvae. They undertook a range of functional tests on the body wall, heart, pharyngeal and visceral muscle in first instar larvae that argued against any general physiological defect in the musculature [9]. It was only in the second instar that *DmiR-1* mutant larvae showed a severe locomotion defect and collapse of the musculature. Intriguingly, food and, by implication, growth appeared to trigger the muscle defect and the lethality. This conclusion is based on an experiment in which larvae were fed just sucrose [9]. In this condition, wild type larvae do not grow, but remain viable and mobile for some two weeks. Strikingly, *DmiR-1* mutants

behave similarly: in the absence of growth, muscle function is indistinguishable from wild-type.

Is the lethal *DmiR-1* phenotype due to loss of *DmiR-1* function in the muscle? Sokol and Ambros [9] addressed this using the Gal4/UAS system to rescue the *DmiR-1* lethal phenotype. They used the 24B Gal4 driver [13], which is often utilised in studies of embryonic development to drive expression in developing muscle. The observed rescue is certainly good evidence for a muscle-specific function of *DmiR-1*, although one cannot exclude the possibility that the Gal4 driver used drives expression in other cell types in larvae, as it does in pupae [14].

Sokol and Ambros [9] argue persuasively that the observed *DmiR-1* phenotype is distinct from that of previously described larval arrest or muscle mutants. However, much of the link between the microRNA and the phenotype remains to be explored. For example, what goes awry in second instar muscle? As the authors point out, very little is known about the rapid growth phase of larval muscle when each fibre grows to around one hundred times its original size [9]. *DmiR-1* may provide an entry point to understanding this fascinating facet of muscle development.

How are the muscle and larval growth phenotypes linked? A straightforward explanation is that compromised muscle function affects larval growth. This is consistent with a relatively subtle first instar muscle phenotype, seen before overt growth effects [9]. Contractions of the body wall muscle are reduced in frequency, and delayed food ingestion indicates weakened pharyngeal muscle. Other explanations are also possible. Indeed, there may be multiple target genes, whose expression is down-regulated by *DmiR-1*, and hence multiple aspects to the phenotype. This highlights that the key to understanding *DmiR-1* function is to uncover its targets. Sokol and Ambros refer to some unpublished work on predicting these targets [9]. The top candidates are apparently not expressed in cells from the mesoderm, and the authors speculate that there may be promiscuous transcription in the very active nuclei of rapidly growing muscles and *DmiR-1*'s role is to post-transcriptionally suppress such detrimental expression.

How is microRNA expression controlled? Genes encoding microRNAs are similar to protein encoding genes in that the primary transcript, which is capped, polyadenylated and can be quite long, is produced by RNA PolII [5]. Consistent with this, early indications are that control of microRNA gene expression may be similar to that of protein encoding genes. For *DmiR-1*, Sokol and Ambros [9] focused on two key regulators of *Drosophila* muscle development, the transcription factors Twist and Mef2 [15]. They found that *DmiR-1* expression is regulated by *twist* and may have input from *mef2*, too. Similarly, major controllers of mammalian muscle have recently been identified as regulators of mouse *miR-1* gene expression [16]. In muscle development, there is still much to be learnt about how its main regulators orchestrate all the events required to form fully functional muscle tissue. This work indicates that microRNAs should now be placed amongst the candidate target genes of these regulators (Figure 1).

miR-1 is a conserved microRNA. What else is known about it in other species? In vertebrates, as in *Drosophila*, it is expressed in developing skeletal and heart muscle [11, 16]. Two functional studies have recently been reported. First, transfection of human *miR-1* into HeLa cells produces a global shift in gene expression towards the profile typical of skeletal and heart muscle [17]. Second, overexpression of *miR-1* in mice reduces cell proliferation during heart development, although any possible skeletal muscle effect has not been reported yet [16]. No effect of *DmiR-1* on the heart has yet been described in *Drosophila* [9].

When the techniques of molecular biology were first applied to Developmental Biology some thirty years ago, there was a lot of attention on post-transcriptional control mechanisms in development. This culminated in many seminal findings, for example the establishment of the *Drosophila* A/P axis and the role of cyclins in early development [18]. In more recent times, interest waned in this aspect of the control of gene expression and its role in development, but the recent work on microRNAs rejuvenates this field.

Most studies of muscle development have understandably centred on traditional areas of Developmental Biology, like patterning and specification. Like some others before them, the work described by Sokol and Ambros [9] instead focuses on the development of a functional musculature. One can anticipate a shift towards this type of study as efforts to advance tissue repair will require a knowledge of how functional tissue assembles, grows and is maintained.

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Sexual Behaviour: Do a Few Dead Neurons Make the Difference?

Why do males and females behave so differently? Sexually dimorphic neural circuitry has just been found in parts of the fly's brain thought to control mating behaviour. Might this explain why males and females have such distinct sexual behaviours?

Jai Y. Yu and Barry J. Dickson

Males and females of most species behave rather differently, particularly when it comes to sex. This makes sexual behaviours attractive models for trying to understand innate behaviours in general. Instead of trying to identify all the genes and all the neurons involved in a given behaviour, and then figure out how they all work, one can just look for the genes and neurons that make the sexes different, and try to understand how these genes and neurons shape the distinct sexual behaviours of males and females. In what might be a major step towards this goal, Kimura *et al.* [1] have now discovered a clear difference in neural circuitry in the brains of male and female fruit flies. This difference, they speculate, might just explain why male flies do the male thing and females do not.

Fly sex is a complicated business. To woo a female, the male must perform an elaborate song-and-dance courtship ritual [2]. The *fruitless* (*fru*) gene, the RNA transcript of which is spliced differently in males and females, plays a key role during development to lay the foundation for this behaviour (Figure 1). In males, *fru* RNA is spliced in such a way as to encode male-specific Fru^M proteins. Males that lack the *fru* gene [3], or splice it the wrong way [4], make a complete mess of the courtship ritual. For the most part, they do not even bother, and if they do, they are just as likely to try to woo another male as a female. What is more, females that splice *fru* RNA in the male way, and therefore make Fru^M, behave like males and try to woo other females [4]. So, genetically, *fru* seems to account for much of the difference between male and female sexual behaviour. Can *fru* also lead us to the neuronal circuits in the brain that make the difference?

It turns out that Fru^M is made in ~3000 neurons in the male brain, or ~3% of the total number of neurons [5]. These neurons are grouped into distinct clusters in various regions of the brain. Are these neurons also present in females, and if so, what is different about them? Because the female *fru* transcripts do not encode Fru^M, it has been rather difficult to identify cells in females that correspond to the Fru^M-expressing cells in males. To circumvent this problem, two groups [6,7] recently used gene targeting to insert coding sequences for an independent marker (GAL4) into the *fru* locus, replacing the alternatively spliced exon so that the marker would be produced in both males and females. Surprisingly, these studies revealed that almost all of the Fru^M-producing neurons in the male have counterparts in the female, and at a gross level, they seem to be wired up the same way. Of course, this does not exclude more subtle differences in neuroanatomy, but without knowing which of these ~3000 neurons make the essential difference, there seemed little point to go on examining them all at higher resolution.

Kimura *et al.* [1] took a different line of attack, both technically and strategically. They isolated a random enhancer trap insertion further downstream in the *fru* locus, called NP21 (Figure 1). NP21 labels many, but not all, of the Fru^M neurons in males, as well as the corresponding cells in females. Kimura *et al.* [1] then went on to characterize some of these neurons at higher resolution, undeterred by the lack of behavioural data to indicate which of them might be the most relevant. Nevertheless, two sets of NP21-positive neurons clearly differed anatomically in males and females (Figure 1). One of these, belonging to the so-called *fru*-mAL cluster [5], particularly attracted their attention.

These neurons seem to serve as a relay between the primary gustatory centre of the brain and higher brain regions thought to integrate information from multiple sensory modalities. There are, on average, about 30 NP21-positive *fru*-mAL cells in males and about five in females. In a